

## REMARKS

### Status of the Application

The COMMUNICATION from the Examiner, paper number 16, mailed September 14, 2001, was in response to Applicants' Response and Amendment mailed July 3, 2001, which was in response to a non-final office action mailed February 6, 2001.

#### *Applicants' response and amendment entered*

The Examiner entered Applicants' response and amendment mailed July 3, 2001, acknowledging that claims 1 to 2 and 12 to 13 were canceled and that claims 14 to 27 are pending and under examination (see first paragraph of page 2 of the COMMUNICATION). Claims 14 to 27 were added as new claims in Applicants' July 3, 2001, response and amendment.

#### *Applicants' last response and the restriction requirement*

In this COMMUNICATION, the Examiner has alleged that Applicants' response and amendment mailed July 3, 2001, is not fully responsive to the previous office action on the merits because all pending claims, which were directed to an enzyme, were canceled, and replaced by newly added methods of making and using claims.

In the Restriction Requirement dated March 16, 2000, the Examiner alleged that the pending claims of the application were directed to three separate and distinct inventions, as follows:

Group I: Claims 1 to 2, drawn to a thermophilic enzyme having beta-glycosidase activity.

Group II: Claims 3 to 7, drawn to DNA sequences encoding the enzyme having beta-glycosidase activity.

Group III: Claims 8 to 11, drawn to a method of hydrolysis of a beta-glycoside comprising using the beta-glycosidase as a catalyst.

Group I was elected in Applicants' response mailed April 13, 2000.

Filing of CPA allows examination of new restriction group

By re-filing this application as a continued prosecution application (CPA) under 37 C.F.R. §1.53(d), Applicants are entitled to have this first submission for this CPA entered and considered on the merits. Filing of a CPA withdraws finality and allows examination of a different restriction group.

Applicants' prior response incorporated in this CPA

In addition to amendments and remarks herein, Applicants respectfully request entry of the amendments and consideration of the remarks set forth in Applicants' response and amendment mailed July 3, 2001.

The Title

A new title that is more clearly indicative of the invention to which the claims (as amended herein) are directed is presented in the instant amendment.

Claims amended, canceled and added in the CPA amendment

In this CPA amendment, claims 14 to 17 and 20 are amended and new claims 28 to 33 are added. In Applicants' response and amendment mailed July 3, 2001, claims 1 to 2 and 12 to 13 were canceled and claims 14 to 27 were added. Thus, after entry of the instant amendment, claims 14 to 33 will be pending and under consideration.

Support for the Claim Amendments

The specification sets forth an extensive description of the invention in the new and amended claims. Support for claims directed to methods for making a  $\beta$ -glycosidase enzyme, wherein the enzyme comprises a tetramer of four subunits, and each subunit is encoded by a nucleic acid comprising a sequence capable of hybridizing to SEQ ID NO:1, or its complement, under various hybridization and wash conditions, can be found, inter alia, on page 3, lines 13 to 29.

### CONCLUSION

In view of the foregoing remarks, it is believed that the all claims pending in this application (after entry of the instant amendment) are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If any additional necessary fee is required, the Commissioner is authorized to deduct such a fee from the undersigned's Deposit Account No. 06-1050. Please credit any overpayments to the above-noted Deposit Account.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at (858) 678-5070.

Respectfully submitted,

Date:

Oct 18, 2007

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Applicant : Ikuo Matsui et al.                      Art Unit : 1652  
Serial No. : 09/369,735                      Examiner : Maryam Monshipouri, Ph.D.  
Filed : August 6, 1999  
Title : THERMOPHILIC ENZYMES HAVING BETA-GLYCOSIDASE  
ACTIVITY

*In The Specification:*

The specification has been amended as follows.

The pending title has been deleted and replaced with

--METHODS FOR MAKING AND USING A THERMOPHILIC ENZYME AS  
A  $\beta$ -GLYCOSIDASE--

*In The Claims:*

Claim 14 has been amended as follows:

14. (Amended) A method for using a thermophilic enzyme as a  $\beta$ -glycosidase, comprising the following steps:

(a) providing an enzyme, wherein the enzyme comprises a sequence as set forth in SEQ ID NO:2 and the enzyme comprises four subunits to form [forms] a tetramer, [in which] wherein each subunit of the tetramer comprises [the amino acid residues of] a sequence as set forth in SEQ ID NO:2; and

(b) contacting the tetrameric enzyme with a substrate[,] under conditions[,] wherein the enzyme functions as a  $\beta$ -glycosidase on the substrate.

Claim 15 has been amended as follows:

15. (Amended) The method of claim 14, wherein the function comprises hydrolysis of a  $\beta$ -glucoside, wherein the  $\beta$ -glucoside comprises [having] a long alkyl chain [(LA- $\beta$ -D-Glcp)].

Claim 16 has been amended as follows:

16. (Amended) The method of claim 15, wherein the long alkyl chain [has] comprises 8 or more carbon atoms.

Claim 17 has been amended as follows:

17. (Amended) The method of claim 14, wherein the enzyme has a high affinity to a  $\beta$ -glucoside [having] comprising a long alkyl chain.

Claim 20 has been amended as follows:

20. (Amended) The method of claim 14, wherein the conditions comprise temperatures selected from the group consisting of 90°C or higher and 100°C or higher.

The following new claims have been added:

--28. (NEW) A method for using a  $\beta$ -glycosidase, comprising the following steps:

(a) providing a  $\beta$ -glycosidase, wherein the  $\beta$ -glycosidase comprises a tetramer of four subunits, and at least one subunit is encoded by a nucleic acid comprising a sequence capable of hybridizing to SEQ ID NO:1, or its complement, under conditions comprising a hybridization step comprising 6xSSC and 50% formamide at 42°C and a washing step comprising 6xSSC and 40% formamide at 25°C, and the  $\beta$ -glycosidase is active at temperatures at or above about 90°C or higher; and

(b) contacting the  $\beta$ -glycosidase with a substrate under conditions wherein the  $\beta$ -glycosidase functions as a  $\beta$ -glycosidase on the substrate.

29. (NEW) The method of claim 28, wherein all four subunits are encoded by a nucleic acid comprising a sequence capable of hybridizing to SEQ ID NO:1, or its complement, under conditions comprising 6xSSC and 40% formamide at 42°C.

30. (NEW) A method for using a  $\beta$ -glycosidase, comprising the following steps:

(a) providing a  $\beta$ -glycosidase, wherein the  $\beta$ -glycosidase comprises a tetramer of four subunits, and each subunit is encoded by a nucleic acid comprising a sequence capable of hybridizing to SEQ ID NO:1, or its complement, under conditions comprising a hybridization step comprising 6xSSC and 40% formamide at 42°C and a washing step comprising 1xSSC and 0% formamide at 55°C, and the  $\beta$ -glycosidase is active at temperatures at or above about 90°C or higher; and

(b) contacting the  $\beta$ -glycosidase with a substrate under conditions wherein the  $\beta$ -glycosidase functions as a  $\beta$ -glycosidase on the substrate.

31. (NEW) A method for using a  $\beta$ -glycosidase, comprising the following steps:

(a) providing a  $\beta$ -glycosidase, wherein the  $\beta$ -glycosidase comprises a tetramer of four subunits, and each subunit is encoded by a nucleic acid comprising a sequence capable of hybridizing to SEQ ID NO:1, or its complement, under conditions comprising a hybridization step comprising 6xSSC and 30% formamide at 42°C and a washing step comprising 0.1xSSC and 0% formamide at 62°C, and the  $\beta$ -glycosidase is active at temperatures at or above about 90°C or higher; and

(b) contacting the  $\beta$ -glycosidase with a substrate under conditions wherein the  $\beta$ -glycosidase functions as a  $\beta$ -glycosidase on the substrate.

32. (NEW) A method for making a  $\beta$ -glycosidase enzyme, comprising the following steps:

(a) providing four subunits of a tetramer, wherein each subunit is encoded by a nucleic acid comprising a sequence capable of hybridizing to SEQ ID NO:1, or its complement, under conditions comprising a hybridization step comprising 6xSSC and 50% formamide at 42°C and a washing step comprising 6xSSC and 40% formamide at

25°C, and the  $\beta$ -glycosidase is active at temperatures at or above about 90°C or higher; and

(b) contacting the four subunits together under conditions wherein they form a tetrameric enzyme comprising a  $\beta$ -glycosidase activity.

33. (NEW) A method for hydrolyzing a  $\beta$ -glycoside, comprising the following steps:

(a) providing a  $\beta$ -glycosidase, wherein the  $\beta$ -glycosidase comprises a tetramer of four subunits, and each subunit is encoded by a nucleic acid comprising a sequence capable of hybridizing to SEQ ID NO:1, or its complement, under conditions comprising a hybridization step comprising 6xSSC and 50% formamide at 42°C and a washing step comprising 6xSSC and 40% formamide at 25°C, and the  $\beta$ -glycosidase is active at temperatures at or above about 90°C or higher; and

(b) contacting the  $\beta$ -glycosidase with a  $\beta$ -glycoside under conditions wherein the  $\beta$ -glycosidase hydrolyzes the  $\beta$ -glycoside.--